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## Cited References

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**Applicant or Patentee** : Dosuk D. Lee *et al.*  
**Serial or Patent No.** : Not Yet Assigned  
**Filed or Issued** : October 20, 2000  
**Title** : Chemotherapeutic Composition Using Nanocrystalline Calcium Phosphate Paste

**VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY STATUS**  
**(37 CFR 1.9(f) and 1.27(c)) - SMALL BUSINESS CONCERN**

I hereby declare that I am

- ☐ the owner of the small business concern identified below:  
☒ an official of the small business concern empowered to act on behalf of the concern identified below:

**Name of Small Business Concern:** Etex Corporation

**Address of Small Business Concern:** 350 Massachusetts Avenue  
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I hereby declare that the above identified small business concern qualifies as a small business concern as defined in 13 CFR 121.12, and reproduced in 37 CFR 1.9(d), for purposes of paying reduced fees to the United States Patent and Trademark Office, in that the number of employees of the concern, including those of its affiliates, does not exceed 500 persons. For purposes of this statement, (1) the number of employees of the business concern is the average over the previous fiscal year of the concern of the persons employed on a full-time, part-time or temporary basis during each of the pay periods of the fiscal year, and (2) concerns are affiliates of each other when either, directly or indirectly, one concern controls or has the power to control the other, or a third party or parties controls or has the power to control both.

I hereby declare that rights under contract or law have been conveyed to and remain with the small business concern identified above with regard to the invention, entitled CHEMOTHERAPEUTIC COMPOSITION USING CALCIUM PHOSPHATE PASTE by inventors Dosuk D. Lee and Maria Aioloa described in:

- ☒ the specification filed herewith.  
☐ application serial no. [\*\*SERIAL NUMBER\*\*], filed [\*\*FILING DATE\*\*].  
☐ patent no. [\*\*PATENT NUMBER\*\*], issued [\*\*ISSUE DATE\*\*].

If the rights held by the above identified small business concern are not exclusive, each individual, concern or organization having rights to the invention is listed below and no rights to the invention are held by any person, other than the inventor, who would not qualify as an independent inventor under 37 CFR 1.9(c) if that person made the invention, or by any concern which would not qualify as a small business concern under 37 CFR 1.9(d), or a nonprofit organization under 37 CFR 1.9(e). NOTE: Separate verified statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities. (37 CFR 1.27)

**Assignee Name:**

**Assignee Address:**

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I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28(b))

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent on which this verified statement is directed.

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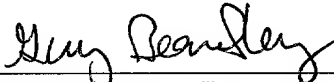
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APPLICATION  
FOR  
UNITED STATES LETTERS PATENT

APPLICANT : Dosuk D. Lee and Maria Aiolova  
TITLE : Chemotherapeutic Composition Using Nanocrystalline  
Calcium Phosphate Paste

**CHEMOTHERAPEUTIC COMPOSITION**  
**USING NANOCRYSTALLINE CALCIUM PHOSPHATE PASTE**

**Cross Reference To Related Applications**

This application is a continuation-in-part application of U.S.S.N.  
09/153,133, filed September 15, 1998 entitled "Calcium Phosphate Delivery  
Vehicle and Adjuvant," the contents of which are incorporated by reference.

This application also is a continuation-in-part application of U.S.S.N.  
08/729,342, filed October 16, 1996, entitled "Delivery Vehicle," the contents of  
which are incorporated by reference.

**Background of the Invention**

The present invention relates to pharmaceutical compositions and  
methods for the treatment of cancer.

Following surgical removal or radiation therapy for the removal or  
reduction of hard and soft tissue tumors, patients are faced with the possibility of  
persistent tumor cells, metastasis and tumor reoccurrence. In the case of bone  
tumors, patients face the additional problem of poor mechanical integrity of the  
bone. Cancer patients typically receive postoperative chemotherapy to reduce the  
chances of tumor reoccurrence and metastasis. Chemotherapy also is used for the  
treatment of inoperable tumors. Systemically delivered anticancer drugs often  
produce severe side effects, such as liver toxicity, cardiotoxicity, hair and weight  
loss. Therapies often are discontinued or otherwise limited due to these adverse  
side effects.

While the effectiveness of chemotherapy has improved tremendously, the side effects associated with its administration remain a significant factor in patient mortality. Therefore, an important consideration when treating bone tumors and soft-tissue tumors with chemotherapeutic agents is maintaining a long-acting, yet highly effective concentration of the anticancer agent at the local site of the tumor while minimizing the often toxic systemic side effects.

MacroMed reports the use of a biodegradable polymer having reverse thermal gelation properties for intra-tumoral injections. Under the tradename OncoGel™, the poly(lactide-co-glycolide)-based polymer is deliverable through a small-gauge (25) needle and localized delivery is reported. See, U.S. Patent No. 5,702,717 for further information on the polymeric material.

Hydroxyapatite is a major mineral in bone and teeth. It demonstrates excellent biocompatibility with bony tissue and has been used in the orthopedic industry as bone-filling material. Anticancer agents such as adriamycin, cis-platin and methotrexate have been incorporated into porous hydroxyapatite beads and blocks, and sustained release of the agents have been demonstrated.

Administration of the drug-loaded blocks to a tumor site in a cancer rat model resulted in increased life span and reduction in body weight loss. See, Yamamura et al. *Jpn. J. Pharmacol.* **65**:289 (1994); Yamamura et al. *Jpn. J. Pharmacol.* **66**:433 (1994); and Uchida et al. *J. Orthop. Res.* **10**(3):440 (1992).

However, hydroxyapatite ceramics typically are dense, highly crystalline materials, and as such, are poorly resorbable. Porosity must be engineered into the material to permit drug uptake during drug loading and drug release at the tumor site. Engineering of the hydroxyapatite block for a particular drug release profile is difficult and not easily reproducible. An additional

limitation of use of a hydroxyapatite solid block or bead is that it requires surgical implantation.

Calcium phosphate cements are compositions having one or more dry components and a liquid which combine to form a material that is capable of setting into a solid calcium phosphate product. Materials that set into solid calcium phosphate mineral products are of particular interest as such products can closely resemble the mineral phase of natural bone, are potentially remodelable, and are biocompatible.

Patents of interest describing calcium phosphate cements include:

4,684,673 to Adachi et al.; 5,037,639 to tung et al., 5,683,461, 5,676,976 and 5,650,176 to Lee et al; 4,108,690, 5,968,253 to Posner et al. and 5,508,342 to Antonucci et al, as well as 4,880,610, 5,047,031, 5,129,905, 5,336,264, 5,053,212, 5,178,845, and 5,580,623 to Constantz et al.; 5,569,442 and 5,571,493 to Fulmer et al.; and 5,496,399; 5,683,667; 5,683,496; and 5,697,981 to Ison et al. Also of interest are WO 96/36562 and WO 97/17285.

Constantz et al., "Skeletal Repair by in Situ Formation of the Mineral Phase of Bone," *Science* (Mar. 24, 1995) 267: 1796-1798, describes a calcium phosphate cement comprising  $\alpha$ -tricalcium phosphate, monocalcium phosphate monohydrate (MCPM), and  $\text{CaCO}_3$ . Also of interest is Otsuka et al. "A Novel Skeletal Drug Delivery System Using Self-Setting Calcium Phosphate Cement. 9: Effects of the Mixing Solution Volume on Anticancer Drug Release from Homogeneous Drug-loaded Cement" *J. Pharm. Sci.* **84**(6):733 (June 1995), which describes a calcium phosphate cement made up of tetracalcium phosphate (TTCP) and dicalcium diphosphate (DCPD) and incorporating the anticancer agent 6-mercaptopurine (6-MP). The addition of 6-MP was reported not to interfere with

the setting properties of the cement; however, the drug release profile from the cement was not acceptable, presumably due to crystallization of the calcium phosphate cement with time. The effectiveness of cement as a delivery system was not established, as only model *in vitro* release studies were reported.

5           A number of these calcium phosphate cements suffer from one or more drawbacks, such as low resorbability, rapid set time, poor flow characteristics and inability to provide controlled release of an active agent. None of the calcium phosphate cements report setting times and material flow characteristics which are amenable to injection or cannulation.

10           Thus, there is a need for an anticancer agent delivery system that combines desirable delivery characteristics (e.g. resorbability, controlled release, biocompatibility) in conjunction with the ability to be injectable, and thus able to administer the therapeutic mixture by syringe or cannula.

15           There remains a need for a drug delivery system that slowly releases an anticancer agent exclusively into the tumor.

          There remains a further need for a drug delivery system that is easy to administer to the tumor site with minimum trauma to the patient.

#### Summary of the Invention

20           The present invention provides a nanocrystalline or poorly crystalline calcium phosphate composition for use in the local treatment of cancer. The composition demonstrates excellent biocompatibility, controlled drug release and ease of administration. The composition readily incorporates the drug with simple mixing and can be administered without invasive surgery by injection or by cannula. The setting and crystallization properties of the composition are not



significantly affected by the addition of a therapeutic agent. The nanocrystalline or poorly crystalline features of the calcium phosphate carrier provide ideal drug delivery and bioresorption profiles.

In one aspect of the invention, a method for treating cancer in a mammal is provided, in which an anticancer composition is administered to a tumor site of the mammal. The composition includes a mixture of an anticancer agent and a calcium phosphate paste, and the paste includes one or more nanocrystalline or poorly crystalline calcium phosphates and a physiologically acceptable fluid. The paste has an injectable or formable consistency at the time of administration and is hardenable at the tumor site.

In one embodiment, each calcium phosphate has a Ca/P ratio of less than or equal to 1.7.

In another embodiment, the anticancer agent is selected from the group consisting of methotrexate, cis-platin, prednisone, hydroxyprogesterone, medroxyprogesterone acetate, megestrol acetate, diethylstilbestrol, testosterone propionate, fluoxymesterone, vinblastine, vincristine, vindesine, daunorubicin, doxorubicin, hydroxyurea, procarbazine, aminoglutethimide, mechlorethamine, cyclophosphamide, mephalan, uracil mustard, chlorambucil, busulfan, carmustine, lomusline, dacarbazine (DTIC, dimethyltriazenomideazolecarboxamide), fluorouracil, 5-fluorouracil, cytarabine, cytosine arabinoside, mercaptopurine, 6-mercaptopurine, tamoxifen, paclitaxel, etoposide, vinorelbine, gemcitabine, leuprolide, flutamide, goserelin acetate, and thioguanine, and mixtures thereof.

In another embodiment, the anticancer composition is administered to the tumor site by cannula or by injection, or the anticancer composition is administrable by cannula or injection more than five minutes after its preparation,

or the anticancer composition is administrable by cannula or injection more than twenty minutes after its preparation.

In still another embodiment, the paste hardens into an apatitic calcium phosphate.

5 In some embodiments, the nanocrystalline or poorly crystalline calcium phosphate paste comprises a calcium phosphate selected from the group consisting of poorly crystalline apatitic (PCA) calcium phosphates (PCA), dicalcium phosphates, such as dicalcium phosphate dihydrate (DCPD) and dicalcium phosphate anhydrous (DCPA), tricalcium phosphates (TCP), monetite, 10 monocalcium phosphate monohydrate (MCPM), heptacalcium phosphate, calcium pyrophosphate, calcium metaphosphate, octacalcium phosphates (OCP), hydroxyapatites (HA), or at least one of the nanocrystalline or poorly crystalline calcium phosphates is a poorly crystalline apatitic calcium phosphate.

15 In some embodiments, each of the one or more nanocrystalline or poorly crystalline calcium phosphates has a calcium to phosphate ratio in the range of 1.0 to 1.67, or in the range of 1.3 to 1.67.

In other embodiments, the nanocrystalline or poorly crystalline calcium phosphate paste has an overall calcium to phosphate ratio in the range of 1.0 to 1.7, or in the range of 1.40 to 1.65.

20 In some embodiments, the nanocrystalline or poorly crystalline calcium phosphate paste comprises a physiologically acceptable fluid in an amount sufficient to produce a paste having injectable or formable consistency.

In still other embodiments, a therapeutically effect amount of anticancer agent is released from the composition for a time greater than one week, or for a 25 time greater than two week, or for a time greater than one month, or for a time

greater than three months.

In yet another embodiment, delivery of the anticancer therapy to the tumor site is sufficient to prevent increase of tumor mass without significant weight loss of the mammal, or delivery of the anticancer therapy to the tumor site is sufficient to result in a decrease in tumor mass without significant weight loss in the mammal.

In still another embodiment, the particle size of the nanocrystalline or poorly crystalline calcium phosphate is selected to provide a desired release kinetic of the anticancer drug.

In another aspect of the invention, an anticancer composition includes a mixture of a physiologically effective amount of an anticancer agent and a calcium phosphate paste including one or more nanocrystalline or poorly crystalline calcium phosphate and a physiologically acceptable fluid. The paste has an injectable or formable consistency at the time of administration and hardenable at the tumor site.

In one embodiment, each calcium phosphate having a Ca/P ratio of less than or equal to 1.7.

In another embodiment, the anticancer agent is selected from the group consisting of methotrexate, cis-platin, prednisone, hydroxyprogesterone, medroxyprogesterone acetate, megestrol acetate, diethylstilbestrol, testosterone propionate, fluoxymesterone, vinblastine, vincristine, vindesine, daunorubicin, doxorubicin, hydroxyurea, procarbazine, aminoglutethimide, mechlorethamine, cyclophosphamide, mephalan, uracil mustard, chlorambucil, busulfan, carmustine, lomusline, dacarbazine (DTIC, dimethyltriazenomideazolecarboxamide), fluorouracil, 5-fluorouracil, cytarabine, cytosine arabinoside, mercaptopurine, 6-

mercaptomurine, tamoxifan, paclitaxel, etoposide, vinorelbine, gemcitabine, leuprolide, flutamide, goserelin acetate, and thioguanine, and mixtures thereof.

In still other embodiments, the nanocrystalline or poorly crystalline calcium phosphate cement comprises a calcium phosphate selected from the group consisting of amorphous calcium phosphate, poorly crystalline apatitic (PCA) calcium phosphates (PCA), dicalcium phosphates, such as dicalcium phosphate dihydrate (DCPD) and dicalcium phosphate anhydrous (DCPA), tricalcium phosphates (TCP), monetite, monocalcium phosphate monohydrate (MCPM), heptacalcium phosphate, calcium pyrophosphate, calcium metaphosphate, octacalcium phosphates (OCP), hydroxyapatites (HA), or at least one of the nanocrystalline or poorly crystalline calcium phosphates is selected from the group consisting of amorphous calcium phosphate and poorly crystalline apatitic calcium phosphate.

In some embodiments, each of the one or more nanocrystalline or poorly crystalline calcium phosphates has a calcium to phosphate ratio in the range of 1.3 to 1.67, or the nanocrystalline or poorly crystalline calcium phosphate paste has an overall calcium to phosphate ratio in the range of 1.0 to 1.7, in the range of 1.0 to 1.67 or in the range of 1.40 to 1.65.

In other embodiments, the anticancer composition is of a consistency administrable to the tumor site by cannula or by injection.

In other embodiments, the nanocrystalline or poorly crystalline calcium phosphate paste comprises a physiologically acceptable fluid in an amount sufficient to produce a paste having injectable or formable consistency for at least five minutes, or for at least twenty minutes.

In still other embodiments, the nanocrystalline or poorly crystalline

calcium phosphate paste is hardenable into an apatitic calcium phosphate.

In yet other embodiments, a therapeutically effect amount of anticancer agent is released from the composition for a time greater than one week, or for a time greater than two week, or for a time greater than one month, or for a time greater than three months.

In still another embodiment, delivery of the anticancer therapy to the tumor site is sufficient to at least prevent increase of tumor mass without significant weight loss of the mammal, or delivery of the anticancer therapy to the tumor site is sufficient to prevent a decrease in tumor mass without significant weight loss in the mammal.

In yet another embodiment, the particle size of the calcium phosphate is selected to provide a desired release kinetic of the anticancer drug.

In still another aspect of the invention, a kit for use in preparing a flowable anticancer composition that remain injectable for at least about 20 minutes is provided. The kit includes dry ingredients having a nanocrystalline or poorly crystalline calcium phosphate and a second calcium phosphate in a proportion of about 1:10 to 10:1 by weight; a physiologically acceptable aqueous lubricant in an amount sufficient to produce a flowable product upon combination with the dry ingredients; and an anticancer agent in an amount ranging from about 0.01 to 10 wt. % of the dry ingredients.

In one embodiment, the kit further includes a means of mixing the dry ingredients and the lubricant, or an injecting means.

By "nanocrystalline calcium phosphate (NCP)" is meant a calcium phosphate solid which exhibits crystalline domains on the order of nanometers or Angstroms. NCP materials may include localized structure based upon well-

known calcium phosphates, such as hydroxyapatite, tricalcium apatite, octacalcium phosphate, etc.; however, long-range order is substantially absent. Exemplary NCP materials based upon hydroxyapatite are found in United States Patent No. 5,783,217.

5 By “poorly crystalline apatitic (PCA) calcium phosphate” is meant a synthetic calcium phosphate of apatitic structure demonstrating only short-range crystallinity. The PCA calcium phosphate is not necessarily restricted to a single calcium phosphate phase, provided it demonstrates the characteristic X-ray diffraction pattern of an apatitic mineral, namely two broad peaks in the region of 10 20-35° with a peak centered at 26° and a second peak centered at 32°. Exemplary PCA calcium phosphate materials are found in U.S.S.N. 08/729,342, hereby incorporated by reference.

#### Brief Description of the Drawing

15 The invention is described with reference to the following drawings which are presented for the purpose of illustration only, which are not intended to be limiting of the invention, and in which:

20 Figure 1 is a plot of the % tumor mass change over time in C3H mice for 25 mg/kg cisplatin dose in calcium phosphate delivery vehicle (▲), calcium phosphate delivery vehicle alone (\*), no treatment (+), and systemic IV administration of cisplatin (-);

Figure 2 is a plot of total body weight over time for C3H mice treated with cisplatin in calcium phosphate delivery vehicle of the invention (◆), and for rats treated with a positive control (intravenous administration) (■);

25 Figure 3 is a plot of the % tumor mass change over time for C3H mice

for 25 mg/kg cisplatin dose in calcium phosphate delivery vehicle (▲), calcium phosphate delivery vehicle alone (\*), no treatment (+), and systemic IV administration of cisplatin (-); and

Figure 4 is a plot of total body weight over time for C3H mice treated with cisplatin in calcium phosphate delivery vehicle of the invention (◆), and for rats treated with a positive control (intravenous administration) (■).

### Description of the Preferred Embodiments

The nanocrystalline calcium phosphate composition of the invention demonstrates excellent biocompatibility, controlled drug release and ease of administration. The composition readily incorporates the drug with simple mixing and can be administered without invasive surgery by injection or by cannula. The flowable material may be injected into a cancellous bone void and the material sets into a solid calcium phosphate product that is capable of withstanding physiological loads, making such products extremely attractive for use in the treatment of bone tumors. The composition may similarly be injected into soft-tissue tumors. The flowable nature of the calcium phosphate composition of the invention allows the mixture to flow around and into the tumor, where it hardens into a calcium phosphate cement. The tumor may be encapsulated thereby in the hardened calcium phosphate cement which confines the cancer cells of the tumor and helps to prevent metastasis.

The calcium phosphate paste may include any nanocrystalline or poorly crystalline calcium phosphate material in which the chemical composition and material properties have been selected to provide the desired flow properties and bioresorption. The desired flow properties include the ability to deliver the

calcium phosphate composition by syringe or cannula. The paste is considered “injectable” if it is capable of passing through a 16 gauge needle. In preferred embodiments, injectability is maintained after storage, for example, for one minute, five minutes, ten minutes and most preferably 30 minutes. Injectability is evaluated by determining the ability to inject 1 cc of paste material through a 16 gauge needle or smaller, with only finger or hand pressure being applied by the user. The material is capable of injection through small gauge needles, e.g., 18-gauge to 25-gauge needles.

The paste is made up of a dry powder component, a liquid component and an anticancer agent.

The dry component includes one or more nanocrystalline calcium phosphates. Each calcium phosphate has a calcium to phosphate (Ca/P) ratio of less than or equal to 1.7, and preferably a Ca/P ratio in the range of 1.0-1.65. Calcium phosphates of interest include, but are not limited to, calcium phosphates such as poorly crystalline apatitic (PCA) calcium phosphates (PCA), dicalcium phosphates, such as dicalcium phosphate dihydrate (DCPD) and dicalcium phosphate anhydrous (DCPA), tricalcium phosphates (e.g.,  $\alpha$ - and  $\beta$ -TCPs), monetite, monocalcium phosphate monohydrate (MCPM), heptacalcium phosphates, calcium pyrophosphates, calcium metaphosphates, octacalcium phosphates (OCP), hydroxyapatites (HA), which are nanocrystalline or poorly crystalline in nature. Other calcium phosphates are described by Driessen (1995), which is hereby incorporated by reference. Carbonated or otherwise substituted versions of these calcium phosphates are also contemplated in the invention. Calcium deficient or poorly crystalline apatitic calcium phosphates having a Ca/P ratio of about 1.0-1.65, preferably 1.0-1.6, or 1.0-1.5 or 1.0-1.4, or 1.50-1.65, and more particularly



1.55-1.65, are preferred. Another preferred calcium phosphate is nanocrystalline calcium phosphate (NCP), in particular a hydroxyapatitic NCP.

The preferred nanocrystalline calcium phosphates, NCP, or PCA calcium phosphate, may be used alone, or in combination with each other, or individually with a second calcium phosphate to form the dry component of the composition. Suitable second calcium phosphates are those which are capable of reacting with the primary calcium phosphate to form a hardened cement. Exemplary calcium phosphates include dicalcium phosphates, such as dicalcium phosphate dihydrate (DCPD) and dicalcium phosphate anhydrous (DCPA), tricalcium phosphates (TCP), monetite, monocalcium phosphate monohydrate (MCPM), heptacalcium phosphate, calcium pyrophosphate, calcium metaphosphate, octacalcium phosphates (OCP), hydroxyapatites (HA). A preferred dry powder combination is a nanocrystalline calcium phosphate and dicalcium phosphate, e.g., DCPD. Another preferred embodiment is a poorly crystalline apatitic calcium phosphate and a dicalcium phosphate. A detailed description of the preparation and characteristics of suitable calcium phosphates can be found in U.S. Patent No. 5,783,217, which is hereby incorporated by reference.

A particularly preferred dry component is a combination of calcium phosphate powders which react to form an apatitic calcium phosphate. Hydroxy apatite is the mineral component of naturally-occurring bone having a Ca/P ratio of 1.67, although the actual Ca/P ratio of bone varies between 1.5 and 1.7. The composition and relative amounts of the dry powder components preferably are selected to provide an overall Ca/P ratio in the range of 1.0-1.7, or 1.3-1.65, or 1.4-1.6, or preferably close to that of naturally-occurring bone, that is in the range of

1.45 to 1.7. It has been observed that calcium-deficient compositions exhibit more rapid, resorption characteristics. In some embodiments where rapid resorption is desired, it may be desired to use select dry powder components to provide an overall Ca/P ratio of less than 1.67, and preferably in the range of 1.4 to 1.65.

5           The composition of the invention also includes one or more anticancer agents. The anticancer agent may be introduced with the powder component, or with the liquid component of the composition, or it may be separately added to the composition. Suitable anticancer agents may be one or more of known chemotherapy drugs such as methotrexate, cis-platin, prednisone, hydroxyprogesterone, medroxyprogesterone acetate, megestrol acetate, diethylstilbestrol, testosterone propionate, fluoxymesterone, vinblastine, vincristine, vindesine, daunorubicin, doxorubicin, hydroxyurea, procarbazine, aminoglutethimide, mechlorethamine, cyclophosphamide, mephalan, uracil mustard, chlorambucil, busulfan, carmustine, lomusline, dacarbazine (DTIC, 10 dimethyltriazenomideazolecarboxamide), fluorouracil, 5-fluorouracil, cytarabine, cytosine arabinoside, mercaptopurine, 6-mercaptopurine, tamoxifen, paclitaxel, etoposide, vinorelbine, gemcitabine, leuprolide, flutamide, goserelin acetate, and thioguanine, and mixtures thereof. 15

Exemplary anticancer agents and the suggested dosage are found in Table 1. The amount of anticancer agent that is present in the cement will be 20 sufficient to provide a composition that at least prevents tumor growth in the region where the composition has been introduced as compared to a control. In preferred embodiments, the amount and effectiveness of the anticancer agent is sufficient to reduce the tumor size or even to substantially eliminate the tumor.

**Table 1.**

Cancer	Drug	Mechanism of action	Dosage	response rate
Breast	tamoxifan	non-steroidal antiestrogen; inhibits binding of estrogen to receptors	20mg/daily (tablet)	as 1 <sup>st</sup> line hormonal therapy; 60% steroid receptor cancers respond
	doxorubicin	inhibits action of topoisomerase II; forms free radicals, binds to membrane	60-75 mg/m <sup>2</sup> IV (every 21 days)	none known
	methotrexate	inhibitor of dihydrofolate reductase; interferes with cellular enzymes	30-40 mg/m <sup>2</sup> IV (once a week)	with cyclophosphamide and 5-fluorouracil, 30-50%)
	cyclophosphamide	alkylating agent; crosslinks DNA and RNA strands, prevents cell division	50-1000 mg/m <sup>2</sup> ; 1x treatment	35% alone, 90% combination therapy
	5-fluorouracil	effects DNA and RNA	500 mg/m <sup>2</sup> , 1xday, repeat 4-5 wk	partial remission 10-20%
	paclitaxol	stabilizes microtubule formation leading to cell death	175 mg/m <sup>2</sup> , (every three weeks)	56-62% response in previously treated patients
small cell lung	etoposide (+ cisplatin)	interferes with DNA synthesis by interacting with topoisomerases II	80 /m <sup>2</sup> for three days	30-35% alone; 50% with cisplatin
non-small cell lung	paclitaxol (+ cisplatin)	interferes with DNA synthesis by interacting with topoisomerases II	80 mg/m <sup>2</sup> for three days	mg34-40% in previously untreated patients
	vinorelbine (+ cisplatin)	antitubulin, causes mitotic arrest in G2 and M phases	30 mg/m <sup>2</sup> for 3 days	33% alone; 28% with cisplatin
	gemcitabine (+ cisplatin)	affects cell undergoing DNA syntheses and blocks progression through G1/S	not given in lungs	not given in lungs
prostate	leuprolide	desensitizes LH-RH receptors and reduces release of hormones	7.5 mg im, 1X month	survival favored for leuprolide/flutamide combination

	goserelin acetate	down regulates LH-RH receptor, medical castration, reduces testosterone	3.6 mg s.c., 1x month	with flutamide 70%
	flutamide	nonsteroid antiandrogen, used with LH-RH analogue	250 mg, every 8 h, orally	70% combination therapy
	diethylestilbestrol	synthetic estrogen	1-3 mg, daily	65%

In other embodiments, two or more anticancer agents are included in the composition. Certain combination therapies have been identified as particularly effective and are found in Table 2. The amount of anticancer agent that is present in the cement will generally range from about 0.01 to 10, usually from about 0.01 to 5.0 and more usually from about 0.01 to 3.0% by weight of the dry ingredients of the cement.

**Table 2.**

Cancer	combination therapy
breast	5-fluorouracil, doxorubicin ( $> 60 \text{ mg/m}^2$ ), cyclophosphamide
	cyclophosphamide, doxorubicin ( $< 60 \text{ mg/m}^2$ ), 5-fluorouracil
	cyclophosphamide, methotrexate, 5-fluorouracil
	doxorubicin, paclitaxol
small cell lung	cisplatin, etoposide
non-small cell lung	cisplatin, paclitaxol
	cisplatin, vinorelbine
	cisplatin, gemcitabin
prostate	leuprolide, flutamide
	gosereline acetate, flutamide

Also provided are kits comprising the subject chemotherapeutic delivery vehicle, where the dry and liquid components may be present in separate containers in the kit, or some of the components may be combined into one container, such as a kit wherein the dry nanocrystalline or poorly crystalline calcium phosphate components are present in a first container and the liquid components are present in a second container. Instructions for use may also be included. The kit preferably includes dry ingredients comprising amorphous calcium phosphate, nanocrystalline or PCA calcium phosphate and a second calcium phosphate in a proportion of about 1:10 to 10:1 by weight, and preferably about 50:50 by weight; a physiologically acceptable aqueous lubricant (liquid component) in an amount sufficient to produce a flowable product upon

combination with said dry ingredients; and an anticancer agent in an amount sufficient to produce the desired pharmaceutical effect and typically ranging from about 0.01 to 10 wt. % of said dry ingredients. Due to the need to carefully titrate the amount of anti-cancer agent based upon patient body mass and tumor size, the agent may be added separately. The agent may be added to the liquid component prior to mixing with the dry component, or it may be added to the injectable paste, once formed.

In preparing the subject calcium phosphate cements for use in the treatment of cancer, the dry components and the liquid components will be combined using any suitable means to produce a homogeneous, flowable paste-like material having the characteristics described above. One suitable means of combining the dry and liquid components is a mortar and pestle, with which the liquid and solid components are mixed to produce the flowable paste. Alternatively, one may employ an automated mixing device. See, United States Patent No. 5,980,482, hereby incorporated by reference, for further description of a suitable mixing device.

Generally, the calcium phosphate composition is formed into an injectable gel or solid nanoparticle paste by the addition of a liquid component or lubricant. The liquid component is a physiologically acceptable liquid, such as but not limited to, water, saline, sodium phosphates, buffer solutions, serum, or tissue culture media. The liquid component may include one or more solutes, selected to buffer the liquid, stabilize the anti-cancer agent, or otherwise modify the properties of the calcium phosphate composition, e.g., modify reaction and hardening times or modify the composition or crystallinity of the product calcium phosphate. For example, sodium phosphate is typically used to accelerate the hardening of the

calcium phosphate paste. The preferred calcium phosphate paste may be designed to absorb, bind, entrap or otherwise contain the anticancer agent. The calcium phosphate cements, paste-like compositions and products are combined with an anticancer agent for its local delivery to a physiological site of interest. The calcium phosphate paste containing the anticancer agent remains formable and injectable at room temperature, facilitating the administration of the composition to a tumor site by injection or by cannula.

The calcium phosphate cement may be selected to set rapidly or slowly at room temperatures. In many instances it will be advantageous to have the calcium phosphate paste set slowly at room temperature, for example, to allow the medical professional adequate time to administer the therapeutic composition to the tumor site. For example, the calcium phosphate paste may remain flowable and injectable at room temperatures for a significant time, e.g., more than 5 minutes, more than 10 minutes, more than 20 minutes, and up to an hour. The calcium phosphate paste should nonetheless set rapidly under physiological conditions, e.g., less than 20 minutes, less than 10 minutes and preferably within 5 minutes. Thus, preferred calcium phosphate compositions exhibit a "dual setting" characteristic in that they are slow to set at about room temperature, e.g., 20-25 °C, yet set rapidly at physiological temperatures e.g., 35-40 °C. In such instances, a composition including PCA calcium phosphate as one of the components may be used.

In those instances where a more rapidly setting cement is desired, accelerators may be added to the composition. Exemplary accelerators include water-soluble sodium salts, such as sodium phosphate, sodium succinate, sodium lactate, sodium chloride, sodium acetate and the like.

Setting times may be determined using well-established standards, such as the Gillmore needle test (ASTM C 266-89 Standard Test Method of Time of Setting of Hydraulic-Cement Paste by Gillmore Needles) and the needle penetration test C 403/C 403M-95 Standard Test Method for Time of Setting of Concrete Mixtures by Penetration Resistance. The effect of the anticancer agent on the setting times may be determined using these tests as well.

Once in place, the paste hardens and releases the anticancer agent into the tumor environment over an extended period of time, relying on a combination of drug solubility and bioerosion of the calcium phosphate delivery vehicle. The calcium phosphate composition may be formulated to be resorbable over a preselected period of time. Calcium phosphate cements may be prepared which resorb within at least about 1 week, within about one month, within about one month and preferably within a year, so that the calcium phosphate cement may release the anticancer agent into their local environment for as long as is needed, depending on the specific cement from which the composition is prepared. Thus, the subject compositions find use as anticancer agent delivery vehicles, i.e. as anticancer agent depots, in which the local delivery of an anticancer agent for an extended period of time is desired. The subject compositions find particular use as local anticancer agent delivery vehicles for bone tissue, particularly cancellous bone tissue.

In most cases, the anticancer compositions are resorbable. Resorbable calcium phosphate cements biodegrade over time, ultimately leaving little or no residual material in the body. Resorbability generally eliminates the need for surgical removal of the delivery vehicle, after completion of chemotherapy.

Resorbable calcium phosphate cements also allow the controlled delivery of active



agents to a the tumor site at a specific rate. The anticancer agent typically is delivered to a tumor site at a rate comparable to the resorption rate. Custom designed resorbability characteristics of the anticancer composition provides for selected delivery rates. In preferred embodiments, weakly resorbing calcium phosphate cements will be used to provide a slow release delivery of the anticancer agent to the tumor site. In other embodiments, the calcium phosphate cement will be strongly resorbable and provide a means to deliver a fast, quick dose of the anticancer agent to the tumor site. In yet other embodiments, a combination of weakly and strongly resorbable calcium phosphates will be used to produce a variable or pulsatile kinetic release. The resorption rate, and therefore the delivery rate, can be adjusted to hours, days, weeks, months, and even years by varying the preparations of the variously resorbing components.

A strongly resorbing calcium phosphate is characterized as follows: when at least 0.1 gram (preferably 0.1-0.5 g) is implanted in an osseous, subcutaneous or intramuscular site, at least 80% of the material is resorbed within one year. In more preferred embodiments, 0.5 gram of the calcium phosphate will be resorbed within nine months, six months, three months, and ideally one month or less, depending of the desired delivery profile desired at the tumor site. Weakly resorbable means that less than 80% of 0.1 gram of starting calcium phosphate is resorbed after one year. Resorption, as used herein, encompasses solubility based dissolution processes, as well as active cellular or enzyme based processes. Preferred materials are resorbed through active cellular or enzymatic processes. By controlling the rate of active degradation of the calcium phosphate cement, the inventive calcium phosphate cements can be tailored to have linear resorption rates, and can be tailored to avoid initial high concentration spikes where

undesirable.

Resorbability of the calcium phosphate vehicles also may be varied through the adjustment of one or more physical parameters including vehicle size, vehicle particle size, porosity, density, and/or crystallinity. For example, monolithic devices, on the order of one gram will resorb more slowly than one gram of the same material when in particulate form. Two or more of these parameters will generally be adjusted in concert to fine-tune the final resorption rate.

For precipitated calcium phosphates, particle sizes may be controlled by careful control of the precipitation rate. Rapid precipitation, followed by rapid harvesting of the precipitate, is useful in the production of small particle sizes (e.g. particle size ranging from 5 nm to 150 nm) of low crystallinity. The use of standard milling processes known to the art (e.g. ball mills, roller mills, jet mills) followed by precise sieving, will also be useful in preparing vehicles of specific size particles. In other instances materials prepared from slurries, as described in the art, will produce useful particle size materials. Particle sizes of less than 1 mm, preferably less than 0.5 mm, are generally preferred for delivery vehicles intended to be resorbed within six months.

Cements can be induced to form particulates during hardening through the use of an emulsifying agent and injection of the cement as an emulsion. Emulsifying agents will be solubilized from the hardened material leaving a macropore matrix. Suitable emulsifying agents include lethicin, dimethicone, and the like.

Density of the hardened calcium phosphate composition also has a significant effect on resorption rates. Different calcium phosphate compositions

result in different density and grain sizes. Higher density or larger grain size, of course, reduce resorption rates. Leachable or biodegradable materials may be incorporated into the paste that may be subsequently removed *in vivo*, e.g., by dissolution or cellular action, so that a porous vehicle results. Suitable additives should of course be biocompatible and physiologically acceptable at the doses required to induce porosity. Other methods of affecting calcium phosphate density are discussed in Driessens et al. in Encyclopedic Handbook of Biomaterials and Bioengineering, Chapter 31, "Calcium Phosphate Bone Cements", Wise (Ed.), Marcel Dekker (1995).

Selection of the particular calcium phosphates may be made to provide a desired resorption rate in the device. Control of the calcium phosphate cement degree of crystallinity and crystal size may be used to affect the overall vehicle resorption rate. For apatitic calcium phosphates with calcium to phosphorous ratios of 1.3-1.75, poorly crystalline forms are believed to resorb more slowly than less crystalline forms, e.g., amorphous calcium phosphates. This may be an advantage in some applications where sustained release over long time periods is desired. For example, in a comparable *in vivo* site, calcium phosphate cements prepared with nanocrystalline calcium phosphate, and in particular with nanocrystalline calcium phosphate and dicalcium phosphate dihydrate, resorb slowly, e.g., over the course of more than one month. Increased resorption rates may be achieved through the production of apatitic calcium phosphates containing lattice defects, such as ionic vacancies or substitutions. Preferred embodiments include carbonated or otherwise calcium deficient apatites, i.e.,  $\text{Ca/P} < 1.67$ , all of which tend to have increased *in vivo* resorption rates. Each type of calcium phosphate cement may be preferred in specific therapeutic settings.

Further guidance for the production of similar such apatitic calcium phosphates can be found in Structure and Chemistry of the Apatites and Other Calcium Orthophosphates, (Elsevier, Amsterdam, 1994, by J.C. Elliott), and the references contained therein, all incorporated herein by reference.

5           The inventive delivery vehicle can be of any porosity that provides the desirable characteristics for anticancer drug delivery. Porosity facilitates both the diffusion of substances to and from the inventive material and, in certain applications, the penetration of cells and cell processes into the material matrix. Accordingly, calcium phosphate cements of lower porosity tend to resorb more slowly in vivo than those of higher porosity; therefore, the greater the porosity, the greater the rate of resorption. In one embodiment of the invention, porosity is increased through the use of a dry mixture of controlled particle size reactants. For example, a reactant with a larger particle size (e.g. 300-500  $\mu\text{m}$ ) will produce a more porous material. Soluble porogens may also be used to control the porosity of the calcium phosphate material.

15           Additionally, certain molecular factors may be incorporated into the vehicle that can be used to affect its resorption rate by influencing the cellular or enzymatic processes that ordinarily mediate vehicle resorption in the body. These incorporated factors are often biologically active molecules or collections thereof, which affect bone metabolic processes, such as the activity of osteoclasts and/or osteoblasts. In other instances the incorporated factors attract or otherwise affect the activity of one or more of macrophages, monocytes, or foreign body giant cells. Such useful factors include: growth factors, enzyme inhibitors, extracellular matrix components, cytokines and the like.

25           Incorporation of factors, which attract or inhibit osteogenic cells and/or

macrophages, can have a significant effect on calcium phosphate cement resorption rate. Thus, incorporation of bone morphogenetic protein into the inventive calcium phosphate cements will lead to more rapid resorption of the vehicle, particularly in soft tissue implant sites. Additionally, factors that attract osteoclasts (e.g. interleukin-1, lymphotoxin, calcitonin, ) may be used to promote degradation of the vehicle. Osteoclast or macrophage activity inhibitors (e.g. neutral phosphate, glucocorticoids, plicamycin, gallium nitrate) may be used to prolong the resorption process. Extracellular matrix components, such as laminen, RDG peptides, collagen, fibronectin may also be included with the calcium phosphate cements. Further guidance regarding specific factors useful in the regulation of calcium phosphate resorption rates can be found in PCT/US97/18528, incorporated by reference herein. Generally, these factors will be incorporated into the inventive calcium phosphate cements as a concentration of less than 20% wt/wt preferably less than 10% and in most embodiments, less than 5%.

In many instances, calcium phosphate cement resorbability is preferred; however, it is not always required or desired. In some embodiments, a calcium phosphate cement that is either weakly resorbable or substantially non-resorbable may be used. A non-resorbable calcium phosphate cement may be used when prolonged chemotherapy is required over a matter of several years. A non-resorbable calcium phosphate cement may also be desirable in cases when the calcium phosphate cement is used additionally as a support matrix for tissue repair or growth, as a treatment for a disease. Non-resorbable calcium phosphate cements can remain in the body without detrimental effects to the host due to their excellent biocompatibility. Alternatively, non-resorbable

calcium phosphate cements may be surgically removed following the desired delivery period. Suitable non-resorbable or weakly resorbable calcium phosphate calcium phosphate cements include those prepared from sintered hydroxyapatite.

Ultimately, resorption rates may be established empirically by using intramuscular or subcutaneous implantation of the calcium phosphate cement in one or more small animal models to assess the exact effect of formulation adjustments on calcium phosphate cement resorption rates. In these model systems, a variety of candidate formulations may be tested simultaneously and resorption rates can be compared at various time points using standard histological, radiographic or other methods known to the art.

The calcium phosphate paste may also be formulated in a manner which enhances its receptiveness to the cancer cells. For example, the pH of the delivery vehicle may be tailored to be particularly receptive to cancer cells. Cancer cells typically produce an environment that is of lower pH (more acidic) than that associated with healthy host cells. The composition of the calcium phosphate paste may be selected to provide a resultant hardened calcium phosphate having a pH which enhances the effectiveness of the anticancer therapy. Practitioners of calcium phosphate chemistry will know to use those calcium phosphates having the least solubility (for longer release times) at the expected pH at physiological temperatures. In most embodiments, the composition is selected for pH stability at neutrality or under slightly acidic conditions. Guidance for preparations of calcium phosphate cements of differing pHs can be found in the solubility isotherm data such as that provided by Brown in "Phase relationships in the ternary system  $\text{CaO-P}_2\text{O}_5\text{-H}_2\text{O}$  at 25 C," *Amer. Ceram. Soc.* **75**:17 (1992) and by J.C. Elliot in "Structure and Chemistry of the Apatites and Other Calcium

Orthophosphates," supra.

The invention also provides a method for the treatment of cancer. The method includes administering an anticancer composition to a tumor site of a patient. The anticancer composition includes a mixture of an anticancer agent in a calcium phosphate paste delivery vehicle. The paste is made up of two or more calcium phosphates and a physiologically acceptable fluid, each calcium phosphate having a Ca/P ratio of less than or equal to 1.7. The paste is injectable or formable at the time of administration which allows the paste to be introduced directly at and into the tumor. The paste then hardens rapidly at the tumor site.

The method of the invention has been investigated for the treatment of breast and prostate cancer, although the methods and compositions described herein may be readily adapted for the treatment of many kinds of cancer.

Example 1. This example illustrates the typical formation of a nanocrystalline calcium phosphate (NCP) gel for use in a drug delivery preparation for delivery of a chemotherapeutic.

A solution of 218 g of disodium  $\text{Na}_2\text{HPO}_4 \cdot 12 \text{H}_2\text{O}$  in 1.2 liters of distilled water and a solution of 70 g of  $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$  in 0.5 liters of distilled water were prepared. The calcium solution was quickly poured into the phosphate solution at room temperature with stirring. Precipitation was immediate and substantially complete. The precipitate was adjusted to pH 6.4 by the addition of sodium hydroxide solution in order to avoid the formation of acidic calcium phosphates. The precipitate was aged at room temperature for 5 minutes prior to filtration. The precipitate was then filtered through a Buchner filter (with a total surface about  $0.1 \text{ m}^2$ ), and was washed by about 3 liters of distilled water. A gel cake of nanocrystalline calcium phosphate obtained on the filter paper.

Example 2. This example illustrates the typical formation of a nanocrystalline calcium phosphate (NCP) powder for use in a drug delivery preparation for delivery of a chemotherapeutic.

The calcium phosphate apatite material was prepared according to example 3 but with the following modifications. The washed precipitate was collected using a spatula and immersed into liquid nitrogen in a 2.5 L container. Following freezing, the container was transferred into a vacuum chamber for 24 hours ( $10^{-1}$  -  $10^{-2}$  torr), until a fine and dry powder was obtained.

Example 3. This example illustrates the preparation of an apatitic calcium phosphate drug delivery agent for delivery of a chemotherapeutic using NCP powder.

NCP was made by combining two solutions at room temperature. Solution A contained 87.6 g calcium nitrate tetrahydrate in 624 mL of distilled water (pH 5.45). Solution B contained 203.7 g dibasic sodium phosphate heptahydrate and 100.0 g sodium bicarbonate in 2000 mL of distilled water. After mixing together for two minutes at room temperature (pH 6.60), 42 mL of 10% wt/wt NaOH solution was added to the reaction mixture over 11 minutes to bring the pH to 7.29. The mixture was filtered by vacuum filtration (650 mL per funnel) and washed four times with 1000 mL of distilled water. After the last wash, the material was covered in liquid nitrogen and lyophilized for 24 hours. After lyophilization, the powder was pushed through a 500  $\mu$ m sieve.

Dicalcium phosphate dihydrate (DCPD) was prepared at room temperature by the rapid addition of solution B (17.1g  $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ ; 0.250 liters distilled water; pH 5.5-6.0) to a stirred solution A (10 g  $\text{H}_2\text{N}_2\text{O}_4\text{P}$ ; 0.5 liters distilled water; pH 7.8). Immediately thereafter, the sample was filtered using filter paper



(0.05 sq. m) with medium filter speed and a vacuum pressure of about  $10^{-2}$  torr. The material formed a thin cake which was washed with about 2 liters of distilled water and then dried at room temperature for 24-72 hours.

The nanocrystalline calcium phosphate material was physically dry-mixed with DCPD at 50:50 weight percent using a mortar and pestle for 3-5 minutes. Water (1 ml/g of mixed material) was then added to the powder mixture to yield a hydrated precursor of paste-like consistency. The amount of  $H_2O$  added varied, depending on whether a thick or thin paste was desired. The paste material was then placed in a moist tissue environment where upon reaching body temperature ( $37^{\circ}C$ ), it hardened into a solid mass. The hardening process could be delayed for several hours by placing it into a refrigerating temperature of  $4^{\circ}C$ .

Example 4. The goal of this study was to use a calcium phosphate material as a delivery vehicle for cisplatin at a mammary tumor site and to evaluate the effectiveness of cisplatin-calcium phosphate delivery system in treating MTGB Mouse cancer tumors in C3H mice.

A protocol was established to administer a two component calcium phosphate delivery vehicle using the material of Example 6 to a tumor interior. The material was tested at two different cisplatin dosage levels and compared against a systemically delivered control. Table 4 includes the protocol used for the mice study (total of 80 athymic nude mice).

**Table 4.**

Group 1: Positive control (Dose 1 - 25 mg/kg IV systemic control); n=12
Group 2: Positive control (Dose 2 - 10 mg/kg IV systemic control); n=12
Group 3: Negative control (no treatment); n=11
Group 4: Dose 1 - 25 mg/kg 50:50 NCP/DCPD inside tumor; n=11
Group 5: Dose 2 - 10 mg/kg 50:50 NCP/DCPD inside tumor; n=11
Group 6: Negative control (50:50 NCP/DCPD only inside tumor); n=12

The mice were C3H mice (approx. 20 g). They were housed in solid bottom mouse cages maintained within a semi-rigid isolator. They were fed an 18% protein, 5% fat rodent laboratory chow fed ad-lib, gamma-irradiated prior to use. Water was sterile filtered and provided in water bottles. Ambient temperature is 72 degrees F +/- 5 degrees with a relative humidity of 50% +/- 20%.

Mice were inoculated subcutaneously in the flank area with  $1 \times 10^6$  tumor cell in 0.1 mL of RPMI culture medium. After tumors had grown to 8-10 mm, 0.1 cc of treatment or control materials (except for positive systemic controls which were administered by IV) were injected directly into the tumor with an 18-gauge needle. The positive control cisplatin solution was prepared by dissolving a specified amount of cisplatin in sterile saline for injection through a 25 gauge needle directly into the tumor. The appropriate calcium phosphate delivery vehicle was supplied as a sterile powder in a preweighed package. At the time of injection, each powder was hydrated with the appropriate amount of saline and loaded into a 0.1 cc syringe for injection through a 16 gauge needle. Each material was mixed on site and immediately injected at the tumor site using a 1 cc syringe

with a Luer-Lok® tip and a 16-gauge needle. At the study site, each animal was weighed and its tumor size was recorded, using hand-held calipers for the measurements. Mice were sacrificed when tumor mass was greater than 10% of body weight.

5           Animals were monitored over a 20-day period for changes in body weight and tumor mass. Tumor shrinkage accompanied by the absence of toxic side effects (as determined by loss of body weight) was considered an indication of successful treatment.

10           Figure 1 is a plot of the % tumor mass change over time for 25 mg/kg cisplatin dose in calcium phosphate delivery vehicle (▲), calcium phosphate delivery vehicle alone (\*), no treatment (+), and systemic IV administration of 25 mg/kg cisplatin (-). As is clearly shown, negative control animals (curves (\*) and (+)), which received no chemotherapy, demonstrated rapid and excessive tumor growth. The animals were sacrificed on Day 7 due to excessive tumor load. The plot clearly demonstrates that the cisplatin locally delivered to the tumor in a calcium phosphate delivery vehicle based on NCP/DCPD matched the ability of intravenous delivery of cisplatin (positive control); however, positive control animals died on Day 5 due to excessive weight loss. See, Figure 2. Positive control animals, however, remained active and apparently healthy over the 20 day observation period.

20           Example 5. The goal of this study was to use a calcium phosphate material as a delivery vehicle for cisplatin at a prostate cancer tumor site and to evaluate the effectiveness of cisplatin-calcium phosphate delivery system in treating DU-145 human cancer tumors in nude mice.

25           A protocol was established to administer a two component calcium

phosphate delivery vehicle using the material of Example 6 to a tumor interior. The material was tested at two different cisplatin dosage levels and compared against a systemically delivered control. Table 5 includes the protocol used for the mice study (total of 36 NU/NU-nuBR nude mice).

**Table 5.**

Group 1: Positive control (Dose 1 - 25 mg/kg IV systemic control); n=6
Group 2: Positive control (Dose 2 - 10 mg/kg IV systemic control); n=6
Group 3: Negative control (no treatment); n=6
Group 4: Dose 1 - 25 mg/kg 50:50 NCP/DCPD inside tumor; n=6
Group 5: Dose 2 - 10 mg/kg 50:50 NCP/DCPD inside tumor; n=6
Group 6: Negative control (50:50 NCP/DCPD only, inside tumor); n=6

The mice were female 5-6 week old Crl:NU/NU-nuBR, which are nude outbred mice. mice (approx. 17-21 g). They were housed in solid bottom mouse cages maintained within a semi-rigid isolator. They were fed a 18% protein, 5% fat rodent laboratory chow fed ad-lib, gamma-irradiated prior to use. Water was sterile filtered and provided in water bottles. Ambient temperature is 72 degrees F +/- 5 degrees with a relative humidity of 50% +/- 20%.

Mice were inoculated subcutaneously in the flank area with  $2 \times 10^6$  tumor cell in 0.1 mL of RPMI culture medium with 30% Matrigel. After tumors had grown to  $100 \pm 35$  mg, 0.1 cc of treatment or control materials (except for positive systemic controls which were administered by IV) were injected directly into the tumor with an 18-gauge needle. The positive control cisplatin solution was prepared by dissolving a specified amount of cisplatin in sterile saline for

injection through a 25 gauge needle directly into the tumor. The appropriate calcium phosphate delivery vehicle was supplied as a sterile powder in a preweighed package. At the time of injection, each powder was hydrated with the appropriate amount of saline and loaded into a 0.1 cc syringe for injection through a 16 gauge needle. Each material was mixed on site and immediately injected at the tumor site using a 1 cc syringe with a Luer-Lok® tip and a 16-gauge needle. At the study site, each animal was weighed and its tumor size was recorded, using hand-held calipers for the measurements. Mice were sacrificed when two consecutive tumor mass measurements greater than 1000 mg were recorded.

Animals were monitored over a 20-plus day period for changes in body weight and tumor mass. Tumor shrinkage accompanied by the absence of toxic side effects (as determined by loss of body weight) was considered an indication of successful treatment.

Figure 3 is a plot of the % tumor mass change over time for (a) 25 mg/kg cisplatin dose in calcium phosphate delivery vehicle, (b) calcium phosphate delivery vehicle alone, (c) no treatment and (d) systemic IV administration of 25 mg/kg cisplatin. As is clearly shown, negative control animals (curve (b)), which received no chemotherapy, demonstrated rapid and excessive tumor growth. The animals were sacrificed on Day 14 due to excessive tumor load. The plot clearly demonstrates that the cisplatin locally delivered to the tumor in a calcium phosphate delivery vehicle based on NCP/DCPD matched the ability of intravenous delivery of cisplatin (positive control) to contain tumor growth; however, positive control animals died on Day 4 due to excessive weight loss. See, Figure 4. Positive control animals, however, remained active and apparently healthy over the 21 day observation period.

[illegible]

1. A method for treating cancer in a mammal, said method comprising:  
administering to a tumor site of the mammal an anticancer composition  
comprising a mixture of an anticancer agent and a calcium phosphate paste, said  
paste comprised of one or more nanocrystalline or poorly crystalline calcium  
5 phosphates and a physiologically acceptable fluid, the paste having an injectable or  
formable consistency at the time of administration and hardenable at the tumor  
site.

2. The method of claim 1, wherein each calcium phosphate having a  
Ca/P ratio of less than or equal to 1.7.

3. The method of claim 1, wherein the anticancer agent is selected from  
the group consisting of methotrexate, cis-platin, prednisone, hydroxyprogesterone,  
medroxyprogesterone acetate, megestrol acetate, diethylstilbestrol, testosterone  
propionate, fluoxymesterone, vinblastine, vincristine, vindesine, daunorubicin,  
doxorubicin, hydroxyurea, procarbazine, aminoglutethimide, mechlorethamine,  
15 cyclophosphamide, mephalan, uracil mustard, chlorambucil, busulfan, carmustine,  
lomusline, dacarbazine (DTIC, dimethyltriazenomideazolecarboxamide),  
fluorouracil, 5-fluorouracil, cytarabine, cytosine arabinoside, mercaptopurine, 6-  
mercaptopurine, tamoxifen, paclitaxel, etoposide, vinorelbine, gemcitabine,  
leuprolide, flutamide, goserelin acetate, and thioguanine, and mixtures thereof.

4. The method of claim 1, wherein the anticancer composition is  
administered to the tumor site by cannula or by injection.

5. The method of claim 4, wherein the anticancer composition is administrable by cannula or injection more than five minutes after its preparation.

6. The method of claim 5, wherein the anticancer composition is administrable by cannula or injection more than twenty minutes after its preparation.

7. The method of claim 1, wherein the paste hardens into an apatitic calcium phosphate.

8. The method of claim 1, wherein the nanocrystalline or poorly crystalline calcium phosphate paste comprises a calcium phosphate selected from the group consisting of poorly crystalline apatitic (PCA) calcium phosphates (PCA), dicalcium phosphates, such as dicalcium phosphate dihydrate (DCPD) and dicalcium phosphate anhydrous (DCPA), tricalcium phosphates (TCP), monetite, monocalcium phosphate monohydrate (MCPM), heptacalcium phosphate, calcium pyrophosphate, calcium metaphosphate, octacalcium phosphates (OCP), hydroxyapatites (HA).

9. The method of claim 8, wherein at least one of the nanocrystalline or poorly crystalline calcium phosphates is a poorly crystalline apatitic calcium phosphate.

10. The method of claim 1, wherein each of the said one or more nanocrystalline or poorly crystalline calcium phosphates has a calcium to



phosphate ratio in the range of 1.0 to 1.67.

11. The method of claim 1 wherein each of the said one or more nanocrystalline or poorly crystalline calcium phosphates has a calcium to phosphate ratio in the range of 1.3 to 1.67.

5 12. The method of claim 1 wherein the nanocrystalline or poorly crystalline calcium phosphate paste has an overall calcium to phosphate ratio in the range of 1.0 to 1.7.

10 13. The method of claim 1, wherein the nanocrystalline or poorly crystalline calcium phosphate paste has an overall calcium to phosphate ratio in the range of 1.40 to 1.65.

14. The method of claim 1, wherein the nanocrystalline or poorly crystalline calcium phosphate paste comprises a physiologically acceptable fluid in an amount sufficient to produce a paste having injectable or formable consistency.

15 15. The method of claim 1, wherein a therapeutically effect amount of anticancer agent is released from the composition for a time greater than one week.

16. The method of claim 1, wherein a therapeutically effect amount of anticancer agent is released from the composition for a time greater than two week.

17. The method of claim 1, wherein a therapeutically effect amount of

anticancer agent is released from the composition for a time greater than one month.

18. The method of claim 1, wherein a therapeutically effect amount of anticancer agent is released from the composition for a time greater than three  
5 months.

19. The method of claim 1, wherein delivery of the anticancer therapy to the tumor site is sufficient to prevent increase of tumor mass without significant weight loss of the mammal.

20. The method of claim 1, wherein delivery of the anticancer therapy to the tumor site is sufficient to result in a decrease in tumor mass without  
10 significant weight loss in the mammal.

21. The method of claim 1, wherein the particle size of the nanocrystalline or poorly crystalline calcium phosphate is selected to provide a  
15 desired release kinetic of the anticancer drug.

22. An anticancer composition comprising a mixture of a physiologically effective amount of an anticancer agent and a calcium phosphate paste, said paste comprised of one or more nanocrystalline or poorly crystalline calcium phosphate and a physiologically acceptable fluid, the paste having an  
20 injectable or formable consistency at the time of administration and hardenable at the tumor site.

23. The composition of claim 22, wherein each calcium phosphate having a Ca/P ratio of less than or equal to 1.7.

24. The composition of claim 22, wherein the anticancer agent is selected from the group consisting of methotrexate, cis-platin, prednisone, hydroxyprogesterone, medroxyprogesterone acetate, megestrol acetate, diethylstilbestrol, testosterone propionate, fluoxymesterone, vinblastine, vincristine, vindesine, daunorubicin, doxorubicin, hydroxyurea, procarbazine, aminoglutethimide, mechlorethamine, cyclophosphamide, mephalan, uracil mustard, chlorambucil, busulfan, carmustine, lomusline, dacarbazine (DTIC, dimethyltriazenomideazolecarboxamide), fluorouracil, 5-fluorouracil, cytarabine, cytosine arabinoside, mercaptopurine, 6-mercaptopurine, tamoxifen, paclitaxel, etoposide, vinorelbine, gemcitabine, leuprolide, flutamide, goserelin acetate, and thioguanine, and mixtures thereof.

25. The composition of claim 22, wherein the anticancer composition is of a consistency administrable to the tumor site by cannula or by injection.

26. The composition of claim 22, wherein the nanocrystalline or poorly crystalline calcium phosphate cement comprises a calcium phosphate selected from the group consisting of amorphous calcium phosphate, poorly crystalline apatitic (PCA) calcium phosphates (PCA), dicalcium phosphates, such as dicalcium phosphate dihydrate (DCPD) and dicalcium phosphate anhydrous (DCPA), tricalcium phosphates (TCP), monetite, monocalcium phosphate monohydrate (MCPM), heptacalcium phosphate, calcium pyrophosphate, calcium

metaphosphate, octacalcium phosphates (OCP), hydroxyapatites (HA).

27. The composition of claim 26, wherein at least one of the nanocrystalline or poorly crystalline calcium phosphates is selected from the group consisting of amorphous calcium phosphate and poorly crystalline apatitic calcium phosphate.

28. The composition of claim 22, wherein each of the said one or more nanocrystalline or poorly crystalline calcium phosphates has a calcium to phosphate ratio in the range of 1.3 to 1.67.

29. The composition of claim 22, wherein the nanocrystalline or poorly crystalline calcium phosphate paste has an overall calcium to phosphate ratio in the range of 1.0 to 1.7.

30. The composition of claim 22, wherein the nanocrystalline or poorly crystalline calcium phosphate paste has an overall calcium to phosphate ratio in the range of 1.0 to 1.67.

31. The composition of claim 22, wherein the nanocrystalline or poorly crystalline calcium phosphate paste has an overall calcium to phosphate ratio in the range of 1.40 to 1.65.

32. The composition of claim 22, wherein nanocrystalline or poorly crystalline calcium phosphate paste comprises a physiologically acceptable fluid in

an amount sufficient to produce a paste having injectable or formable consistency for at least five minutes.

33. The composition of claim 22, wherein nanocrystalline or poorly crystalline calcium phosphate paste comprises a physiologically acceptable fluid in an amount sufficient to produce a paste having injectable or formable consistency for at least twenty minutes.

34. The composition of claim 22, wherein the nanocrystalline or poorly crystalline calcium phosphate paste is hardenable into an apatitic calcium phosphate.

35. The composition of claim 22, wherein a therapeutically effect amount of anticancer agent is released from the composition for a time greater than one week.

36. The composition of claim 22, wherein a therapeutically effect amount of anticancer agent is released from the composition for a time greater than two week.

37. The composition of claim 22, wherein a therapeutically effect amount of anticancer agent is released from the composition for a time greater than one month.

38. The composition of claim 22, wherein a therapeutically effect

amount of anticancer agent is released from the composition for a time greater than three months.

39. The composition of claim 22, wherein delivery of the anticancer therapy to the tumor site is sufficient to at least prevent increase of tumor mass without significant weight loss of the mammal.

40. The composition of claim 22, wherein delivery of the anticancer therapy to the tumor site is sufficient to prevent a decrease in tumor mass without significant weight loss in the mammal.

41. The composition of claim 22, wherein the particle size of the calcium phosphate is selected to provide a desired release kinetic of the anticancer drug.

42. A kit for use in preparing a flowable anticancer composition that remain injectable for at least about 20 minutes, said kit comprising:

dry ingredients comprising a nanocrystalline or poorly crystalline calcium phosphate and a second calcium phosphate in a proportion of about 1:10 to 10:1 by weight;

a physiologically acceptable aqueous lubricant in an amount sufficient to produce a flowable product upon combination with said dry ingredients; and

an anticancer agent in an amount ranging from about 0.01 to 10 wt. % of said dry ingredients.

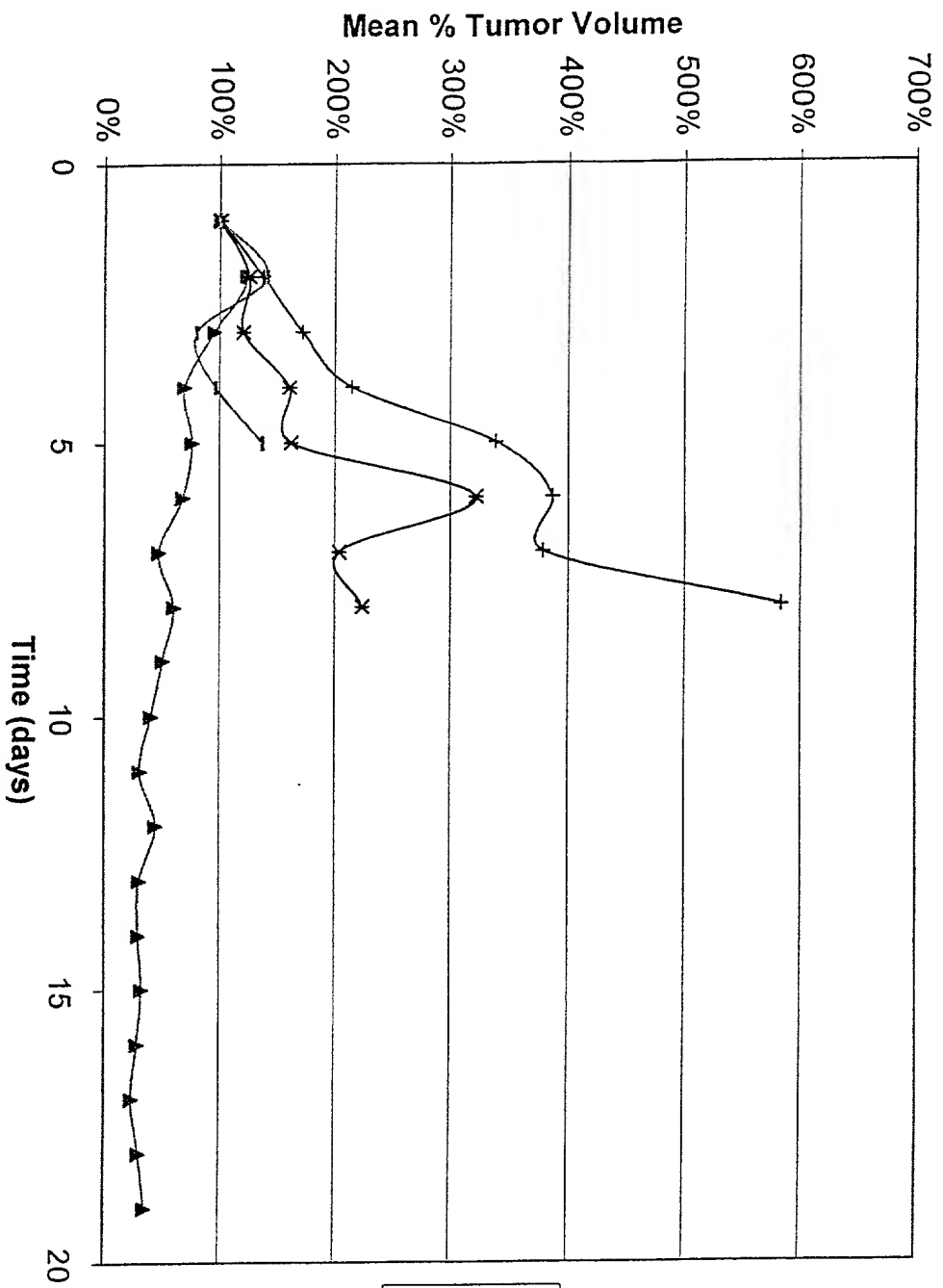


**CHEMOTHERAPEUTIC COMPOSITION**  
**USING NANOCRYSTALLINE CALCIUM PHOSPHATE PASTE**

**Abstract of the Disclosure**

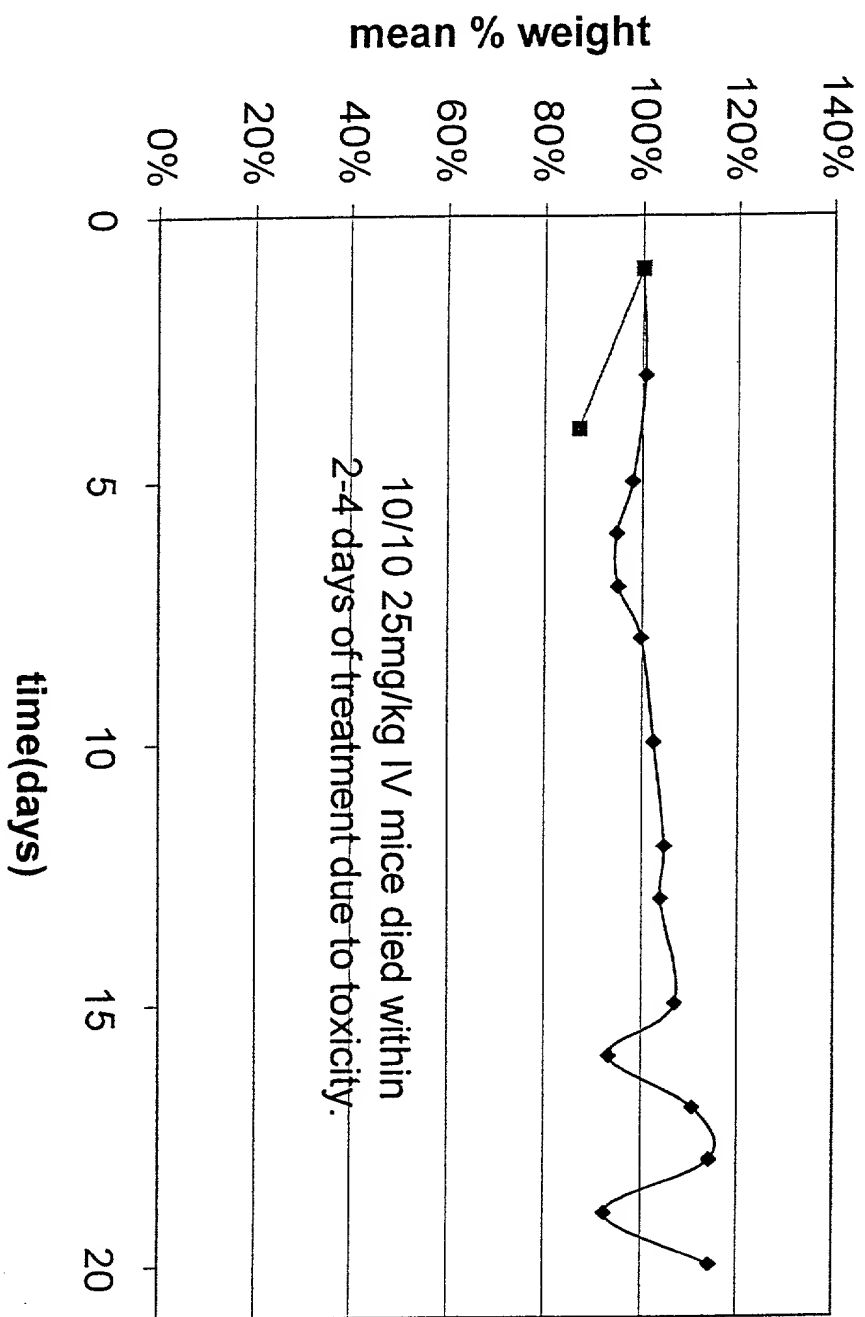
A method and composition are provided for treating cancer in a mammal. The method includes administering to a tumor site of the mammal an anticancer composition comprising a mixture of an anticancer agent and a nanocrystalline or poorly crystalline calcium phosphate paste, said paste comprised of one or more calcium phosphates and a physiologically acceptable fluid, the paste having an injectable or formable consistency at the time of administration and hardenable at the tumor





▲ drug + CaP carrier  
 \* CaP carrier  
 + negative control  
 — drug only (iv)

Figure 1



—◆— drug + CaP carrier  
 —■— drug only (IV)

Figure 2

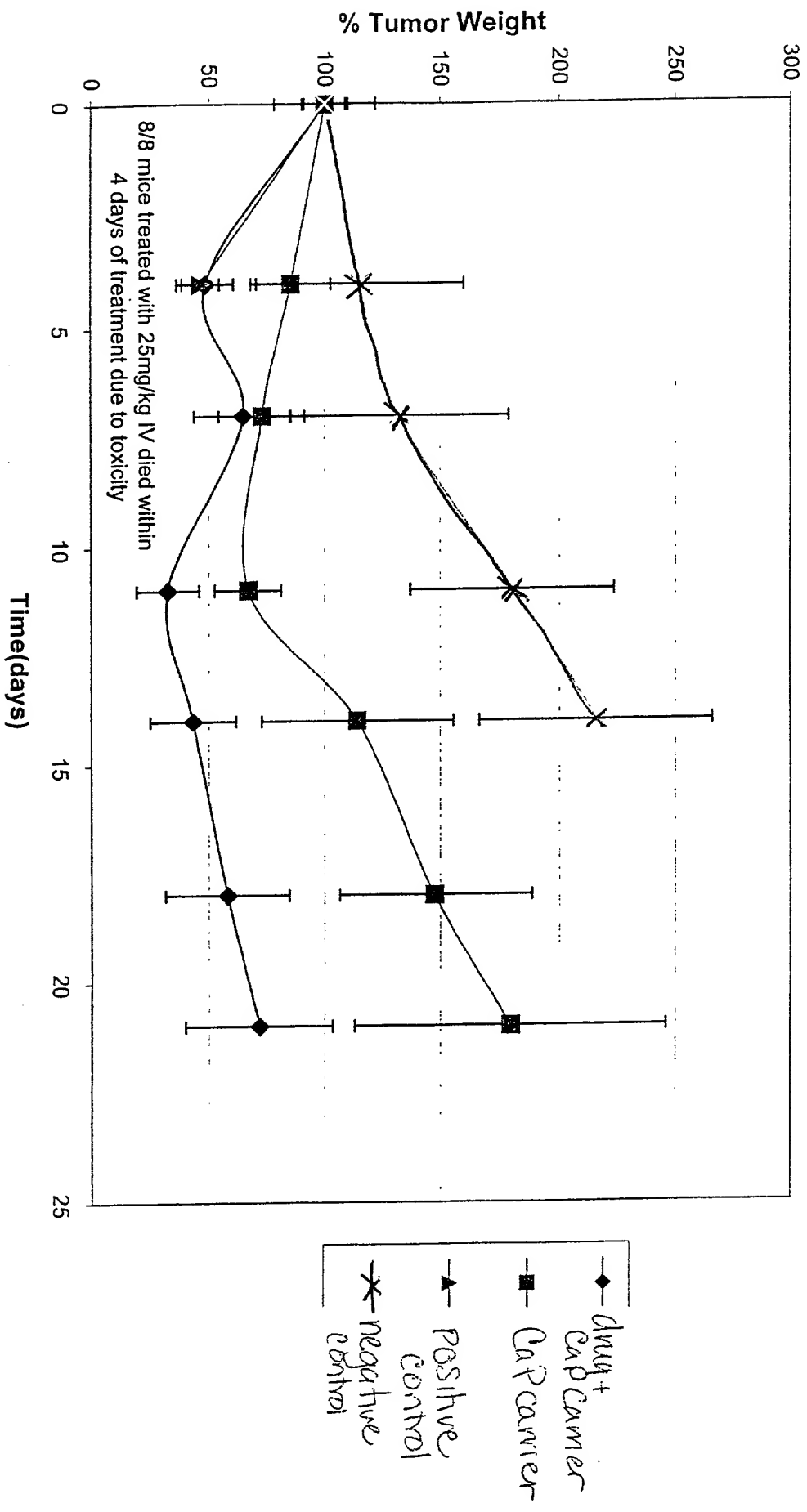


Figure 3



**COMBINED DECLARATION AND POWER OF ATTORNEY**

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled Chemotherapeutic Composition Using Calcium Phosphate Paste, the specification of which

☒ is attached hereto.

☐ was filed on \_\_\_\_\_ as Application Serial No. \_\_\_\_\_  
and was amended on \_\_\_\_\_.

☐ was described and claimed in PCT International Application No. \_\_\_\_\_  
filed on \_\_\_\_\_ and as amended under PCT Article 19 on \_\_\_\_\_.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose all information I know to be material to patentability in accordance with Title 37, Code of Federal Regulations, §1.56(a).

**FOREIGN PRIORITY RIGHTS:** I hereby claim foreign priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed:

Country	Serial Number	Filing Date	Priority Claimed?
			Yes/No

**PROVISIONAL PRIORITY RIGHTS:** I hereby claim priority benefits under Title 35, United States Code, §119(e) and §120 of any United States provisional patent application(s) listed below filed by an inventor or inventors on the same subject matter as the present application and having a filing date before that of the application(s) of which priority is claimed:

Serial Number	Filing Date	Status

## COMBINED DECLARATION AND POWER OF ATTORNEY

**NON-PROVISIONAL PRIORITY RIGHTS:** I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose all information I know to be material to patentability as defined in Title 37, Code of Federal Regulations, §1.56(a) which became available between the filing date of the prior application and the national or PCT international filing date of this application:

Serial Number	Filing Date	Status
09/153,133	September 15, 1998	Pending
08/729,342	October 16, 1996	Pending

I hereby appoint the following attorneys and/or agents to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith: Paul T. Clark, Reg. No. 30,162, Karen L. Elbing, Ph.D., Reg. No. 35,238, Kristina Bieker-Brady, Ph.D., Reg. No. 39,109, Susan M. Michaud, Ph.D., Reg. No. 42,885, Mary Rose Scozzafava, Ph.D., Reg. No. 36,268, James D. DeCamp, Ph.D., Reg. No. 43,580, Sean J. Edman, Reg. No. 42,506, Timothy J. Douros, Reg. No. 41,716.

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patents issued thereon.

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